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JANUARY, 1941

Editorial

PROGRESS

A NEW YEAR has dawned. We extend greetings to all our readers, sure in the knowledge and filled with confidence that the veterinary profession will play its important part in carrying the country to victory. It is fitting at this time to examine the work we are called upon to undertake. Veterinary surgeons have for long plied their art mainly in the treatment of animals suffering from the various ailments to which they are subject and in the prevention of some of the contagious disease throughout the country. While we can claim that we have given a good account of ourselves and can point with pride and satisfaction to the suppression of serious diseases, epizootic in character, a survey of the losses to the country due primarily to the presence of other preventible disease causes us to wonder if the veterinary profession is being used to the maximum extent, especially in these critical times. Surveying the disease situation in general we are forced to the conclusion that more and more can be done if, in the control of such diseases, we make fuller use of

methods now well established and used in other countries. The time has come when we must control disease. The country demands it. The maximum amount of milk and meat must be produced at home and the animal feeding stuffs must be used to the best advantage. The maintaining of diseased animals can no longer be allowed if the country is to put forward its greatest effort. We as a profession must accept the responsibility of ridding the country of unprofitable animals and this we willingly agree to do, provided we are given the opportunities to put into operation measures which we believe to be efficient.

There are, in fact, two distinct problems to be faced. One refers to the needs of the country now and in the immediate future, and the other to our needs after world peace has again been restored. To-day as never before the country depends on its milk and meat supply and it is imperative that they be increased to their maximum capacity. There are many factors which limit the increase, not the least of which is disease in our flocks and herds. Organised methods to combat disease are essential. Any schemes which will yield early results are to be encouraged and put into operation with the least possible delay. By organising the profession now efforts can be made to minimise greatly the losses due to disease. We look forward with every confidence that 1941 will see much of the preventible disease under control, the non-productive animals removed from the country's flocks and herds, and the veterinary profession, every member of it, fully occupied in carrying out an organised scheme whereby their knowledge and art are being used to the full.

The second point we must bear in mind is that in this and other countries there is bound to occur an increased demand for livestock: when peaceful times are again with us it will be necessary to build up these stocks. Many of us recall vividly the chaotic position 20 years ago when the demand for poultry exceeded all the supplies and how diseased stock was disseminated throughout the land causing ruin to individuals and giving rise to a deterioration in the health of fowls throughout the country of such a nature that we have not yet fully recovered. We must now be forewarned and it is imperative that such a state of affairs is not repeated. There is now much more knowledge concerning disease control at our command. Let us put it all into practice so that good, sound, healthy foundation stocks of animals of all kinds will be at our command to restock the farms at home and abroad when the demand arises.

General Articles

THE STANDARDISATION OF JOHNIN

1. The Suitability of the Guinea-Pig as a Test Animal

By R. E. GLOVER

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Of recent years considerable progress has been made in the elaboration of johnins of good potency for the diagnosis of Johne's disease in cattle. Investigations into the value of products prepared in various types of media and of different batches of johnin have been hampered, however, by the lack of a suitable small laboratory animal which can be sensitised with *Mycobact. paratuberculosis* and used for the comparative assay of different johnins in a manner similar to the intracutaneous tuberculin test in tuberculous guinea-pigs. Potency tests in cattle are undoubtedly of considerable value and in the hands of British workers (Dunkin, etc.) have yielded valuable information on differences in activity of successive batches of johnin. The disadvantage of the test in the bovine species, quite apart from the difficulty in maintaining adequate numbers of infected cattle for the purpose, depends upon the moderate sensitivity of most Johne-infected animals comparable to the relatively low plane of sensitivity of tuberculous cattle to tuberculin.

In a recent series of experiments (Buxton and Glover, 1939), an attempt was made to induce a uniformly high grade of activity to johnin by injecting cattle subcutaneously with suspensions of living Johne bacilli in gum acacia and liquid paraffin. The animals became moderately allergic, but their sensitivity was relatively fleeting. Similar results were obtained in the goat: in this species an effort was made to reinforce a waning allergy by repeated injections of Johne bacilli in oil, but although a temporary stimulation was achieved, the results were not entirely satisfactory.

The object of the present investigation was to determine whether the guinea-pig could be rendered sufficiently sensitive to enable comparative johnin tests to be performed. It has already been established that guinea-pigs injected with *Mycobact. avium* respond to avian tuberculin (Crawford, etc.), and this test has been used with some success to determine the potency of tuberculin of this type. It is evident that the considerable resistance of the guinea-pig to infection by *M. avium* and the absence of gross lesions in inoculated animals does not prevent the development of a skin sensitivity. There would

seem, therefore, to be no *a priori* reason why the guinea-pig should not be used for a similar purpose in regard to johnin, in spite of the generally accepted opinion that it is completely refractory to infection.

Early attempts at sensitising guinea-pigs with aqueous suspensions of Johne bacilli given by the subcutaneous, intramuscular, intraperitoneal or intravenous routes were not encouraging and will not be reported in detail. Occasionally it was found that a batch of guinea-pigs inoculated by one of these routes had become sufficiently sensitised to enable comparative tests to be carried out with fairly satisfactory results, but it proved impossible to secure a uniform degree of sensitivity in successive groups of animals. Irregularities were also experienced when the organisms, in oily substrates such as olive oil, liquid paraffin, paraffin wax, butter, etc., with and without a previous coating of gum acacia, were injected intramuscularly or subcutaneously. A great improvement was effected when the organisms were taken up in liquid paraffin prior to injection by the intraperitoneal route, and the results of a series of tests which have now been completed have shown that the guinea-pig can be rendered reasonably allergic by this means.

It is claimed that the intradermal tuberculin test in the tuberculous guinea-pig is capable of determining variations in the value of tuberculins ranging from 20 per cent. to 50 per cent. above or below a given standard (generally International Standard tuberculin). It was not anticipated that equally fine shades of difference would be detectable by the johnin test in guinea-pigs, but numerous tests have shown that relatively small changes in potency of different johnins can be recognised by the intradermal injection of suitable serial dilutions.

Sensitisation of the Guinea-Pig

With the exception of one experiment, which will be described later, the guinea-pigs have invariably been sensitised with the strain known as "teps." This culture was used by Dunkin (1928, 1933) in most of his experiments and is described as a mixture of three strains of *M. paratuberculosis* (1928).

It was found that a considerable weight of bacillary bodies was necessary in order to induce a reasonable level of allergy and it proved difficult to obtain the necessary amounts from growths on solid medium. The "teps" strain has been trained, however, to grow on Henley's synthetic medium without phlei (Dunkin, 1933) or on a modified Long's medium (Glover, 1932). The former fluid was used for these experiments. A fragment of a young film was used to initiate a fresh culture of the organism in Erlenmeyer flasks. Under suitable conditions the surface of the liquid was usually completely covered in from four to six weeks. The thin crust was lifted with a glass spatula, weighed and transferred to a mortar where it was reduced to a creamy suspension by thorough grinding. Sterile liquid paraffin, warmed to 45 deg. C., was then slowly added until a quantity sufficient to make a suspension contain-

ing 60 mg. per ml. had been obtained. Each guinea-pig was injected with 0.5 ml. by the intraperitoneal route.

It was found that in most cases the guinea-pigs gained in weight: those which did not continue to develop were excluded from the tests as such animals were generally poor reactors. A preliminary test was applied about the 45th day. Two guinea-pigs were epilated and eight dilutions of a heat-concentrated johnin, ranging from 1 : 1,000 to 1 : 18,000, were injected intradermally in the flank, using a dose of 0.2 ml. Readings were taken at the 24th and 48th hours. If this orientating experiment proved satisfactory the main test was performed on the remaining animals in the group.

It was noticed that following the application of a multiple test of this nature, some of the animals showed a marked loss of condition which was not observed in untreated animals. An occasional death occurred at this stage. At autopsy, the peritoneal cavity frequently contained a large excess of a clear ascitic fluid. The mesentery was thickened and loops of small intestine caught up in the intense inflammatory reaction which had arisen, were adherent to one another or were fixed to the abdominal wall. The liver and spleen were slightly enlarged and showed on their surfaces irregular fibrous bands or foci. The gastro-splenic omentum was always thickened and beset with firm yellow or white nodules containing a thick caseo-pus very rich in acid-fast bacilli, while firm, well-circumscribed foci were seen in the liver and spleen. Occasionally the tracheo-bronchial glands were enlarged while hæmorrhagic lesions were present in the lungs. Control guinea-pigs inoculated with Johne bacilli in oil but not subjected to multiple intradermal tests showed lesions of a similar nature, but they were usually much less extensive. It is considered probable that the intradermal johnin tests evoked some degree of systemic reaction which accelerated the progress of the lesions in the peritoneal cavity. The impression has been formed that the incorporation of the Johne bacilli in the liquid paraffin facilitates the spread of the organism through the peritoneal cavity and that the tissues react in a more violent manner. Whether this is the explanation of the enhanced sensitivity of guinea-pigs is not easily proved, but the evidence certainly suggests that the combination of the organism with liquid paraffin appreciably increases the sensitising effect.

In the course of this experiment a certain number of guinea-pigs was injected intraperitoneally with liquid paraffin alone. This induced a slight generalised fibrinous reaction of the mesentery and omentum but, as might have been expected, the animals were completely insensitive to johnin.

Johnins Used for Guinea-Pig Tests

In the case of tuberculin it is possible to assess the potency of a preparation of unknown value by comparison with a selected "standard" such as the sample of "O.T." kept at the National Institute for Medical Research, Hamp-

stead, or with a PPD, which has been carefully matched with it. The problem of evaluating johnins is more complicated, since there is no " standard " which can be used for the differential assay of unknown products.

Up to the present johnins used in the field have been tested by the intradermal injection of suitable dilutions in positive and negative cattle. Such trials have shown that successive batches of johnins may show appreciable variations in their capacity to produce a readable reaction in allergic animals, but little information is as yet available as to the amount of the active principle which is likely to detect the maximum proportion of Johne-infected cattle without inducing non-specific and misleading reactions in animals which are free from the disease. Under these circumstances the choice of any particular preparation or strength of the active principle as a " standard " must be purely arbitrary.

For the purpose of this investigation a quantity of a heat concentrated, synthetic medium johnin was prepared. The " teps " strain was seeded on to 12 flasks of Henley's synthetic medium without *M. phlei*. After 3½ months' incubation at 39.5 deg. C. the cultures were steamed for 1½ hours, filtered and made up to the original volume with distilled water. Glycerol was then added in the proportion of 12.0 ml. per litre, and the fluid was evaporated on a water bath to 1/10th its initial volume. The fluid thus obtained was passed through a Berkefeld candle while hot, and preserved with merthiolate in a strength of 1 : 25,000. This preparation will be referred to as J.15 and has been used as a standard throughout this and subsequent investigations.

TABLE 1

Tests in Negative Cattle

Johnin	Number of Cow			
	129	133	165	166
J.15	*7 : 9 : 10	7 : 9 : 9	10 : 11 : 13	7 : 8 : 10
J.17	7 : 8 : 9	6 : 8 : 9	10 : 12 : 13	5 : 6 : 7
J.52	6 : 7 : 8	6 : 6 : 7	9 : 10 : 11	6 : 7 : 9
J.53	6 : 7 : 7	6 : 8 : 8	9 : 10 : 11	6 : 8 : 9

* Figures represent: (1) initial skin fold measurement in mm.; (2) 48-hour reading; (3) 72-hour reading.

Three other johnins were also available, viz., J.17, J.52 and J.53. They were prepared in a manner similar to J.15 with the exception that J.53 was concentrated to 1/5 its original volume. It will be noted that glycerol was mixed with the final cultures prior to evaporation. The necessity for the addition of glycerol to liquid cultures of *M. tuberculosis* on synthetic medium to replace the considerable quantities which are used by the organism during

the period of growth has been stressed by Douglas and Hartley (1934). Seibert (1934) has also recommended the incorporation of glycerol in tuberculin prior to concentration by dialysis as a first stage in the preparation of PPD. It is not yet known how much glycerol should be added to johnin. Certain experiments, which are not yet complete, have shown that this component is broken down during the growth of Johne bacilli on synthetic medium and it was therefore considered desirable to add a small amount of the polyalcohol before concentrating by heat in order to stabilise the final product. In the present investigation an addition of 1.2 ml. of glycerol was made to each 100 ml. of original culture fluid. Further investigation may show that this quantity does not represent the optimum proportion.

TABLE 2

Tests in Positive Cattle

Johnin	Dilution	Cow No. 146			Cow No. 155		
J.15	undil.	7 : 12 : 20	D	+	9 : 13 : 18	D	+
	1 : 5	7 : 10 : 15	D	+	9 : 11 : 14	D	+
	1 : 10	8 : 10 : 12	SD	+	9 : 11 : 13	SD	+
	1 : 20	8 : 9 : 11		—	9 : 9 : 11		—
J.17	undil.	7 : 13 : 19	D	+	8 : 14 : 19	D	+
	1 : 5	8 : 12 : 14	SD	+	10 : 16 : 18	D	+
	1 : 10	7 : 10 : 14	SD	+	9 : 10 : 13	SD	+
	1 : 20	8 : 10 : 12	?	?	9 : 10 : 12		—
J.52	undil.	8 : 12 : 20	D	+	8 : 13 : 16	D	+
	1 : 5	8 : 11 : 16	D	+	9 : 13 : 15	D	+
	1 : 10	6 : 7 : 11	SD	+	10 : 12 : 14	SD	+
	1 : 20	7 : 8 : 10		—	9 : 11 : 12	?	?
J.53	undil.	8 : 10 : 17	D	+	7 : 10 : 13	SD	+
	1 : 5	8 : 10 : 12	SD	+	9 : 11 : 13	?	?
	1 : 10	7 : 9 : 9		—	9 : 10 : 11		—
	1 : 20	8 : 10 : 10		—	9 : 10 : 10		—

D = Diffuse
SD = Slightly diffuse

The preliminary tests of johnins 15, 17, 52 and 53 were undertaken in a series of four negative and two positive cattle, with the results shown in Tables 1 and 2. In the negatives, the four preparations were quite satisfactory, all producing pea-like reactions with an average increase in skin measurement not

exceeding 3 mm. at the 72nd hour. In the positive cattle three of the johnins (15, 17 and 52) gave almost identical results, while 53 was apparently slightly lower in potency.

Two of these johnins (15 and 17) were then tested in a small herd which was known to be affected with Johne's disease. Of the 16 animals tested 9 were unequivocably negative, 6 were positive to both johnins and one was doubtful to one johnin (15) and negative to the other (17). Information was thus obtained that the johnins were capable of evoking allergic reactions in cattle presumed to be affected with Johne's disease.

Intradermal Tests in Normal Guinea-Pigs with Heat Concentrated Johnin

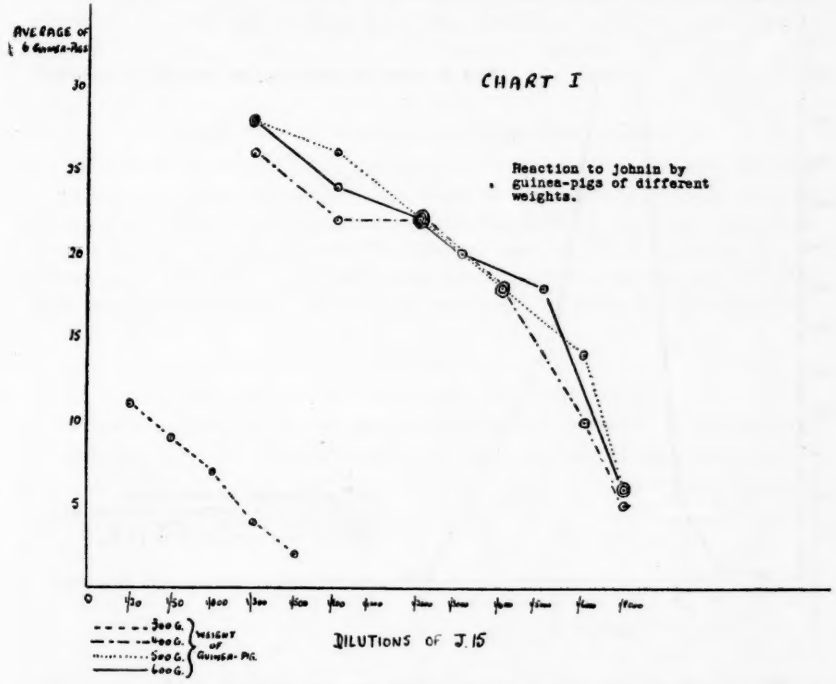
The heat-concentrated johnins described above were tested on a series of normal guinea-pigs. Fifteen animals were injected intradermally with the product undiluted and diluted 1 : 5, 1 : 10 and 1 : 20. A dose of 0.2 ml. of the neat fluid gave rise to a slightly hæmorrhagic plaque measuring from 3 mm. to 5 mm. in diameter. The reaction reached its maximum at the 24th hour, rapidly subsided and had nearly disappeared by the 48th hour. No difference could be detected between J.17, J.52 and J.53, but J.15 gave a slightly larger reaction which in five of the animals persisted until the third day. A 1 in 5 dilution of all the johnins was without effect on the normal guinea-pig skin.

Response of Guinea-Pigs of Varying Age and Period of Maximum Sensitivity

It proved impossible to obtain satisfactory skin tests in small guinea-pigs. In the early experiments animals weighing from 250 to 300 g. were sensitised with suspensions of Johne bacilli in oil and were tested at from four to eight weeks later. The results were not only highly irregular but reactions were rarely observed with dilutions of johnin of 1 : 200 and over. Bunney and Gottschall (1938) have recently claimed that adult guinea-pigs prepared with small doses of tubercle bacilli are much more sensitive to tuberculin than animals of lower weight. It has been noted in this investigation that the susceptibility of large guinea-pigs to johnin is so much higher that readable reactions are provoked by dilutions varying from 1 : 1,000 to 1 : 8,000. In assessing the results, the criteria of Parish and Okell (1929) have been accepted, viz., that evidence of a reaction is furnished by the highest dilution giving a well-developed positive reaction approximately 8 mm. in diameter. In the case of johnin tests in guinea-pigs, 24-hour readings proved misleading on account of the tardy development of the response to high dilutions, and much more uniform and reproducible figures were obtained by delaying the inspection until the 48th hour after injection.

The substantial advance in sensitivity which follows the increase in weight

of the guinea-pig is shown in Chart 1. Four groups of six animals each (average weight 300 g., 400 g., 500 g., and 600 g. respectively) were sensitised with " teps " and tested seven weeks later with serial dilutions of J.15. It will be noted that in the small animals the dose response was restricted to the narrow range of 1 : 20 up to 1 : 300 whereas the sensitivity of the larger animals was spread over a much wider band. Thus, in good-sized guinea-pigs dilutions of 1 : 500 up to 1 : 2,000 invariably produce large swellings with deep purple hæmorrhagic centres which become frankly necrotic on the second

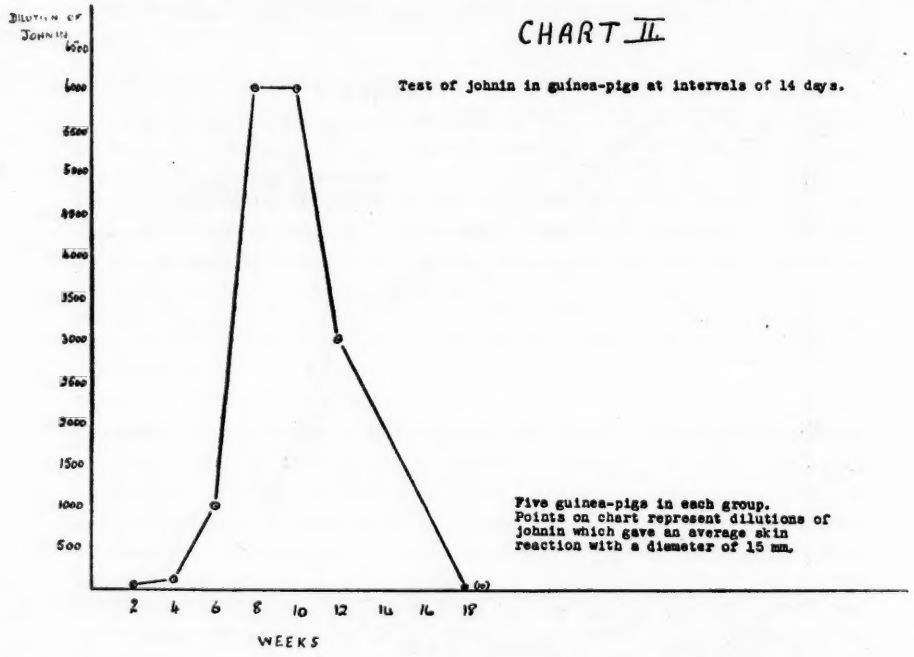


or third day. Higher dilutions give rise to smaller reactions of a roseate nature without necrosis. Between the 1 : 2,000 and 1 : 5,000 range there is a steady decline in sensitivity and it is in this region that slight differences in potency are most readily detected. The curve tends to flatten out beyond the 5,000 mark.

It has been observed that a small proportion of sensitised pigs, even of the larger size, are not fully allergic and an adequate number of animals must, therefore, be included in each test to permit the elimination of those which are not sufficiently responsive. For crucial tests on the potency of johnins

it is suggested that guinea-pigs weighing from 400 g. to 500 g. should be used.

In connection with tests in guinea-pigs sensitised with a suitable dose of the tubercle bacillus, it is generally considered that the most striking results are obtained from three to four weeks after injection. This is not the case in guinea-pigs prepared with Johne bacilli since the allergic response develops at a slower rate. An appreciable degree of sensitivity is certainly apparent at the fourth week when reactions are obtainable to dilutions of 1 : 400 to



1 : 600 of johnin, but this does not represent the period of maximum sensitivity. The comparatively slow development of allergy was determined by testing series of guinea-pigs at spaced intervals. The animals injected with Johne bacilli in oil were divided into seven groups of five animals which were tested with dilutions of johnin (J.15) ranging from 1 : 50 to 1 : 6,000 at intervals of 14 days, with the exception of the final test which was performed at the 18th week. The results are depicted in Chart 2. The curve represents the average end-point of the reaction at the stated intervals. For this purpose it was decided that the diameters of the skin reactions produced by each dilution should be averaged, and the end-point accepted as that dilution which

gave a mean reaction of 15 mm. in diameter. It will be noted that the graph climbs steadily between the 4th and 6th weeks, remains at a high level until the 10th week and then falls fairly rapidly. At the 18th week 53 animals out of 75 were completely negative to a 1 : 40 dilution. The extent of the reaction was roughly proportional to the strength of the johnin. As might have been anticipated, the responses to low dilutions (1/50 to 1/1,000) in highly sensitive animals were approximately equal in intensity, i.e., a large plaque measuring from 20 mm. to 28 mm. in diameter with an intensely hæmorrhagic or necrotic centre was produced. With higher dilutions the degree of reaction diminished inversely as the dilution until a point was reached at which a small papular swelling of from 6 mm. to 8 mm. in diameter was the sole evidence of a response.

Serial Tests of Guinea-Pigs with the Same Johnin

The batch of johnin (J.15) selected as "standard" was tested on three occasions in groups of sensitised pigs using the same serial dilutions in phosphate citrate buffer. Six animals of approximately the same weight were used at each test and the positions of the dilutions were varied from guinea-pig to guinea-pig. The tests were made seven weeks after the animals had received their sensitising injection. The results are shown in Table 3. It will be seen

TABLE 3

Test of J.15 in Three Groups of Guinea-Pigs

Dilution of johnin	Size of reaction in mm. : average of six guinea-pigs		
	12-6-40	2-7-40	21-8-40
1 : 500	23 × 25	26 × 28	26 × 28
1 : 1,000	16 × 18	18 × 20	20 × 20
1 : 2,000	14 × 14	12 × 14	14 × 16
1 : 4,000	10 × 12	10 × 12	12 × 12
1 : 6,000	8 × 8	5 × 6	7 × 7
1 : 8,000	4 × 6	tr.	4 × 6
1 : 10,000	—	—	—

that in general a reproducible end-point was obtained. With the lower dilutions intense reactions were produced, but as the amount of active principle decreased the skin response became less marked. At the first test the recorded end-point was 1 : 8,000, at the second occasion 1 : 6,000, while the third test was the same as the first.

An attempt was also made to determine to what extent the guinea-pig test was capable of revealing differences in the potency of johnins. A group of 18 sensitised animals was tested with two johnins (15 and 17), and the same johnins diluted to 75 per cent., 50 per cent. and 20 per cent. respectively of

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their original strength. The guinea-pigs were divided into three equal groups, one of which was assigned to a dilution of 1 : 2,000 of these johnins, the second was tested at a 1 : 5,000 level and the third at 1 : 8,000. The 48th hour readings are recorded in Table 4.

TABLE 4

Comparison of Dilutions of the same Johnins (15 *and* 17)

Dilution of johnin	undil.	75%	50%	20%	undil.	75%	50%	20%
	J.15				J.17			
1 : 2,000	25 × 20	25 × 30	15 × 15	15 × 10	25 × 25	25 × 25	15 × 10	10 × 5
1 : 5,000	15 × 10	10 × 10	5 × 5	tr.	18 × 15	15 × 15	10 × 10	—
1 : 8,000	5 × 5	tr.	—	—	tr.	5 × 5	—	—

Each measurement represents the mean skin reaction of six guinea-pigs.

The method of reading the results described by Okell and Parish (1927) for tuberculin was used. "The tuberculin was compared with dilutions of itself in order to determine what percentage difference could be revealed by the test. The reactions were read blind, i.e., the dilutions used were not known and were afterwards compared with the actual value. . . . If the tuberculins differed 40 per cent. or more in potency it was nearly always possible to place them in their correct order by the test."

The johnin test revealed that the intensity of the reactions were directly proportional to the strength of the johnins. It was not possible to detect any difference between undiluted johnin and the 75 per cent. dilutions, but in both instances the two 50 per cent. dilutions were placed intermediate between the strong preparations and the 20 per cent. dilutions. Sufficient experience of the guinea-pig test has now been gained to enable a drop of 50 per cent. or more in potency to be detected without difficulty.

Potency Test of Five Johnins in the Guinea-Pig

The four johnins prepared from the "teps" strain (J.15, J.17, J.52, J.53) which had been used for the preliminary trials in cattle (see page 7) were also tested in the sensitised guinea-pig. In addition, a fifth johnin (J.55) was included: it was obtained by adapting a strain of Johne bacillus (125), isolated from the mesenteric gland of an affected animal in 1938 to grow on Henley's synthetic medium. In conformity with the experience of other workers, the early cultures were very sparse and slow growing, but after repeated passage at intervals of 14 days to three weeks, the fifth generation produced a reasonable film in three months, although the weight of culture was considerably less than that produced by the "teps" strain, under similar conditions. After filtration the fluid was concentrated by heat to 1/10 its original volume.

Eighteen sensitised guinea-pigs were available for the test: they were

divided into three groups of six, one batch being assigned to a dilution of 1 : 2,000, a second to a dilution of 1 : 5,000, and a third to 1 : 8,000. In addition to the five johnins at full strength, J.15, J.17 and J.52 were also reduced to 50 per cent. of their original value. The results of the guinea-pig tests are shown in Table 5. The reactions to J.15, J.17 and J.52 were practically identical in dilutions of 1 : 2,000 and 1 : 5,000, but at 1 : 8,000 J.52 fell away to a slight extent. J.53 and J.55 were not so active as the other three, but they were indistinguishable from the 50 per cent. dilutions of these three johnins. The lower value of J.53 as revealed by this test confirms the general impression which was gained from the trials in cattle and was undoubtedly due to the fact that it was less concentrated. The diminished activity of J.55 may have been attributable to the employment of a strain of the organism differing from the one used to sensitise the guinea-pigs, but it is more likely that, owing to the poor growth on the medium, the amount of active principle elaborated was smaller.

TABLE 5

Comparative Test of Five Johnins

Dilution of johnin	J.15	J.15 50%	J.17	J.17 50%	J.52	J.52 50%	J.53	J.55
1 : 2,000	28 × 26	18 × 18	24 × 24	18 × 16	26 × 24	16 × 16	20 × 18	16 × 16
1 : 5,000	20 × 18	10 × 10	18 × 18	10 × 8	20 × 18	8 × 8	10 × 8	8 × 8
1 : 8,000	5 × 5	tr.	5 × 5	—	—	—	—	—

Each measurement represents the mean skin reaction of six guinea-pigs.

Consideration had to be given, however, to the possibility that guinea-pigs sensitised with the " teps " strain might be more responsive to the homologous product. A group of eight guinea-pigs was therefore injected with 30 mg. of " teps " in liquid paraffin, while a second group of equal size received a similar dose of the strain 125 mentioned above. Cross tests with the two johnins in suitable dilutions were then applied after an interval of seven weeks. The results gave no indication whatsoever that either strain was capable of evoking a higher level of sensitivity to its homologous johnin than to the heterologous product.

Discussion

The fundamental observations of Eagleton and Baxter (1923) and other workers on the intracutaneous tuberculin test in the guinea-pig form the basis of modern methods of standardising tuberculin for human use. In the hands of experienced investigators it is possible by means of this test to detect with confidence differences in the potency of a known standard tuberculin when compared with preparations the value of which is unknown. The accuracy of such tests depends partly on the use of guinea-pigs which have been properly

sensitised and are in a stage of full allergy, and partly on the selection of such dilutions of the tuberculins as will fall on the steepest portion of the dose response curve. There is considerable evidence favouring the view that tuberculins which are destined to be used in cattle should also be tested in this species.

With regard to johnins, the need for preparations of proved potency is self-evident. There are clear indications, however, that cattle affected with Johne's disease are in general less responsive to johnin injected intradermally than are tuberculous animals to tuberculin and it is, therefore, all the more important that johnins used in practice should be of adequate potency. It is only of recent years that methods of preparing satisfactory johnins have been placed on a firm basis and as yet no johnin has been selected which can be universally accepted as a "standard" against which unknown preparations can be checked. The fact that johnin lags behind tuberculin in this respect is largely due to the unsatisfactory nature of tests in small animals. The experiments of Dunkin and others have shown that when applied to johnin-sensitive cattle, serial dilutions can detect certain levels in the difference in potency of johnins. It is probable, however, that these tests are of a gross character and reveal differences in value which are relatively wide. In the absence of more accurate methods, tests in cattle must be regarded as an essential part of an assay on the suitability of johnins for use in the field, but it is doubtful whether a definite standard preparation could be accepted solely on the results of comparative trials carried out in cattle.

The present investigation has shown that the guinea-pig can be sensitised so that it will give satisfactory skin reactions to johnin. The procedure adopted is similar to one suggested by Hagan and Levine (1932) and by Mohler (1939) in which the organisms are incorporated in liquid paraffin and injected intraperitoneally, although these authors have not made a special study of the allergic reactions of guinea-pigs prepared in this manner. It has been found that provided animals of good weight are used, results are obtained which compare very favourably with the intracutaneous tuberculin test. Readable reactions are obtainable with dilutions varying from 1-2,000 up to 1-8,000 and are as easily recorded as tuberculin reactions provided the inspection is made at the 48th hour after injection. The response is not quite specific since guinea-pigs sensitised in this manner also react to tuberculin but only to much lower dilutions. Although scarcely germane to the present discussion, it may be noted, in passing, that guinea-pigs injected intraperitoneally with tubercle bacilli in liquid paraffin become very sensitive to tuberculin but also react to some extent to johnin. It would appear that the incorporation of the organisms in liquid paraffin tends to accentuate the reaction to heterologous products. This possibility has received confirmation in other experiments carried out in cattle (Buxton and Glover, 1939) in which it was demonstrated that animals injected with Johne bacilli in paraffin oil were rendered highly

sensitive to johnin. During the first few weeks following injection the animals were not only highly allergic to johnin but also gave definite reactions to bovine tuberculin. It was evident, therefore, that some degree of cross-reaction was to be expected during the period of maximum sensitivity. As the allergic response declined, however, the skin sensitivity to the bovine product decreased in intensity and eventually the beasts became completely negative, whereas reactions to johnin persisted until the termination of the experiment.

It is freely admitted that insufficient evidence is as yet available as to the precise connection between johnin tests in guinea-pigs and in cattle. The number of cows available was too small to permit any extensive comparisons, but it can be said that, as far as they went, the tests indicated that johnins which gave good reactions in guinea-pigs were also satisfactory when applied to infected cattle. The results are certainly sufficiently encouraging to warrant further trials.

The johnins used in this study were concentrated by heat. Equally promising results, which have been obtained with johnin protein of a PPD variety, will be described in a subsequent paper.

Conclusions

1. Guinea-pigs injected intraperitoneally with cultures of Johne bacilli suspended in liquid paraffin became skin sensitive to heat-concentrated, synthetic medium johnins.

2. The selection of guinea-pigs weighing 400 gm. or more was essential in order to obtain the maximum degree of sensitivity, which reached its peak at about the eighth week after injection. The intensity of the reactions was directly proportional to the strength of the johnins.

3. A reproducible end-point was obtained when tests were applied to successive groups of guinea-pigs sensitised in an identical manner.

4. It was possible to detect a 50 per cent. variation in the potency of different johnins.

5. The values of four johnins as revealed by the guinea-pig test were correlated with the results obtained by injecting suitable dilutions of the same johnins into allergic cattle.

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RIBOFLAVIN DEFICIENCY IN POULTRY

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THE importance of the vitamin B_2 complex in the nutrition of poultry has been extensively studied in the United States. It has been shown that at least three of the component factors are essential for normal health and development of the chicken, viz.:

Riboflavin, necessary for embryonic development, growth and the prevention of leg weakness (so-called curled toe paralysis).
Pantothenic Acid (Filtrate Factor),[1] chick anti-dermatitis factor.[2]
Bó (Pyridoxine),[3] necessary for growth of chicks and maintenance of weight in adult birds.

No mention has been made in English veterinary literature of the occurrence of riboflavin deficiency in chickens. The object of this article is to give an account of the knowledge which has been accumulated and to present some data which indicates that riboflavin deficiency occurs and is of economic importance in this country.

Riboflavin Deficiency in Growing Chickens (Curled Toe Paralysis)

In carrying out feeding experiments Hogan, Shrewsbury and Kempter[4] noted that 11 per cent. of their chickens developed a leg weakness. From the evidence submitted it appears probable that this was in part the result of riboflavin deficiency.

Norris, Henser and Wilgus[5] described a disease in which there occurred poor growth and a paralysis affecting the legs and feet. They showed that this could be prevented by including milk in the ration, and concluded that the value of milk was due to the presence of a hitherto undescribed vitamin. These observations were confirmed by Bethke, Record and Kennard.[6]

Lepkovsky and Jukes[7] and Bethke *et al.*[8] showed that supplemental feeding of flavin to chicks maintained on a ration low in vitamin B_2 prevented the onset of nutritional leg weakness.

Symptoms

The onset is usually sudden. It is noticed that the affected birds have difficulty in using their legs. They tend to sink down on their hocks so that the shank is flat on the ground. The leg weakness becomes more obvious if the birds are forced to run around the pen a few times. As the foot comes into contact with the ground the toes twist inwards and together (Fig. 1). If the leg weakness progresses the birds become unable to lift their hocks from the ground so that they walk on their shanks. At this stage the toes are flexed

and twisted inwards and together; hence the name "curled toe paralysis" has been applied. The twisting of the toes is a symptom of diagnostic value.

In attempts to induce the deficiency experimentally it has been noted that the growth rate was poor, but that normal growth took place when riboflavin supplements were given.

As recovery is taking place some birds will appear to stand on their toes with their hocks fully extended, so that the tibeo-tarsus and tarso-metatarsus form almost a straight line.

Symptoms indicating involvement of the central nervous system are entirely lacking. There is no loss of equilibrium. Although the bird may be unable to lift its hocks from the ground there is no tendency to topple over and struggle on its side. This is an important point in differentiating riboflavin deficiency from chick encephalomalacia.

Incidence and Course

Curled toe paralysis appears to be restricted to young growing chickens. Most cases develop between the third and tenth week after hatching, provided the chicks are normal at the time of hatching. The peak incidence is the third to the sixth week.

The incidence is highest in chicks reared in battery brooders and is less frequent in those reared in solid floor houses. It appears to be uncommon in chicks that have access to grass runs. Norris *et al.*[9] records that the incidence in their birds varied between 20 and 50 per cent.

No figures regarding mortality have been published, but it is probable that the most frequent cause of death is trampling and injury inflicted by other birds in the pen. Whether or not recovery takes place depends upon the extent of nerve damage. In early cases the administration of yeast, milk or riboflavin is usually followed by recovery in two to four days. If no treatment is carried out and the bird survives, a complete or partial recovery takes place but the toes may remain crooked.

Post-Mortem

The gross and microscopic lesions have been studied by Phillips and Engel,[10, 11] who described the condition as a neuro-malacia. They state that severe cases showed marked hypertrophy and softening of the peripheral nerves. The involvement was most frequent and most pronounced in the sciatic nerves, the diameter of which might be as much as four to five times greater than normal. They observed an œdema and yellowish colouration of the nerve. In hypertrophic nerves the fibres are widely separated by an accumulation of metabolic products within the myelin sheath. There was myelin degeneration and axis cylinder swelling and fragmentation. The cells of the sheath of Schwann were increased in number. Myelin degeneration

could be demonstrated in the spinal cord. There was no muscular dystrophy, but nerve endings in the thigh muscles were swollen, bulbous and degenerated.

Personal Observations

During the spring and summer, 1940, a total of 32 chickens exhibiting symptoms similar to those described above were obtained. Fourteen were housed in a solid floor pen and were maintained on the food they had been previously receiving. Nine were given 50 gamma* riboflavin daily. In seven, recovery was complete within three days, one died and one showed no improvement after eight days, when it was killed. The remaining five received no treatment; two died and three slowly recovered in an average of seven days.

Eight of the above 32 chickens were obtained from a group of 53 birds during the fourth week after hatching. They had been fed on a commercial starter mash of which the formula was not known. Yeast was added to the remainder of the mash to produce a final content of 3 per cent. Following the feeding of this mixture no further cases developed among the remaining 45 birds.

The lesions in the birds which I have examined resemble closely those already described. The enlargement appears to be most marked in early acute cases. A nerve, if affected, is enlarged uniformly throughout its length. The lesions are bilaterally symmetrical. The yellow colour mentioned by Phillips and Engel was not found in affected Light Sussex chicks, i.e., white-skinned birds, but was present in Cambar and White Leghorns, i.e., yellow-skinned breeds.

On microscopic examination it is noted that the increase in size is principally due to swelling of the myelin substance. There is an increase in cells which possess a large oval pale-staining nucleus. These are presumably those of the neurolemma. Myelin degeneration can be demonstrated by use of the polarising microscope and by Marchi's method of staining. There are no recognisable lesions in the brain.

Differential Diagnosis

(A) *Fowl Paralysis.*

Although curled toe paralysis has some similarities to fowl paralysis there should be little difficulty in distinguishing the two conditions. The typical nerve involvement in fowl paralysis is seldom found in chickens under eight weeks of age, whereas curled toe paralysis is restricted to birds of brooder age. The gait in riboflavin deficiency is quite characteristic and the weakness in both legs is of equal severity. In both diseases the peripheral nerves may be enlarged, but in curled toe paralysis the enlargement extends along the

* Gamma = one-thousandth of a milligram.

FIG. 1.—Day-old chick showing curled toe paralysis.

FIG. 2.—Sciatic nerve of Riboflavin deficient chick. The fibres are swollen and the cellular reaction scanty. X 65.

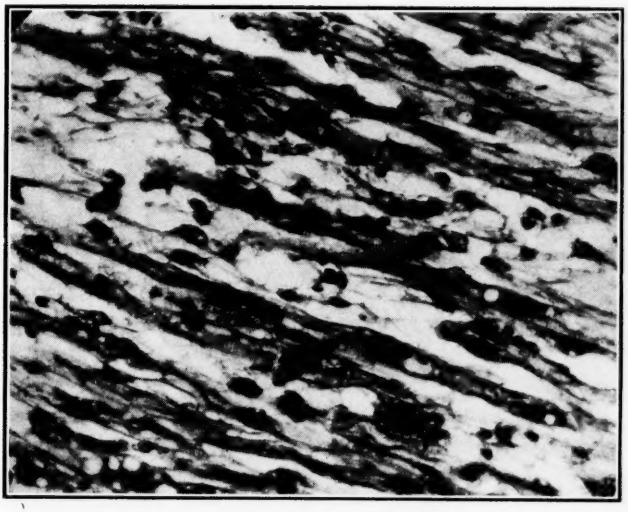

FIG. 3.—Sciatic nerve. X 300.

FIG. 4.—Sciatic nerve of chicken affected with Fowl Paralysis. X 300.

whole length of the nerve and is bilaterally symmetrical. This is seldom the case in fowl paralysis.

On histological examination the difference is obvious. In fowl paralysis the increase in nerve volume is due to an infiltration by small densely staining round cells, whereas in riboflavin deficiency the increase in size is due principally to swelling of the myelin substance and œdema. (Figs. 2, 3 and 4.)

(B) *Rickets.*

The soft beak, nodular enlargement of the costochondral articulations and weak resilient bones of rickets are absent in chicks deficient in riboflavin.

(C) *Perosis or Slipped Tendon.*

Examination of the hocks readily differentiates the condition. The Achille's tendon of one or both legs slips from its groove and produces gross deformity. The hock appears to be swollen. On palpation the displaced tendon can be recognised.

(D) *Encephalomalacia or Crazy Chick Disease.*

This is a disease of young chicks which has recently been recognised in England.[34] The symptoms are of sudden onset. There is loss of equilibrium and somersaulting and the affected birds become unable to stand. They lie on their side, making vigorous propulsive movements of the legs. Inco-ordination is usually progressive and is followed by death. On post-mortem examination the brain is found to be the site of visible lesions. The cerebellum is most frequently affected and is swollen, œdematous, hæmorrhagic and necrotic.

(E) *Epidemic Tremor.*

The existence of epidemic tremor in this country has not yet been confirmed. It appears to be spreading through the Eastern States of America and has recently been recorded in Australia.[12] The age incidence corresponds to that of riboflavin deficiency. Locomotor ataxia may be a prominent symptom. A feature is a fine rapid tremor particularly affecting the head and neck; this is exaggerated on stimulation and is reduced when the bird is at rest. Diagnosis depends upon the demonstration of the causal virus and of microscopic lesions in the brain and visceral organs.

Quantitative Riboflavin Requirements

The quantity of riboflavin required by the young chicken for normal growth has been studied by Norris *et al.*[18] and by Henser, Wilgus and Norris.[14] They made the following observations:

The ration for chicks should contain approximately the following units of

riboflavin per 100 grams of feed for the second to the eighth week, respectively: 350, 290, 240, 200, 160, 130, 100. The total riboflavin consumed per week increased until five weeks of age, after which there was a decrease to the eighth week. It appears that the riboflavin requirement for growth is much greater than the requirement for maintenance. More riboflavin per unit of grain appears to be necessary when the bird is growing rapidly.

Riboflavin Content of Foodstuffs

Most natural foodstuffs contain riboflavin, but the quantity in cereals and oil seeds is insufficient for growing birds. The following are the riboflavin contents of some common poultry foods as quoted by Fixsen and Roscoe.[16] The quantity is expressed in gamma per 100 grams of the food. Whole wheat, 20 gamma; wheat germ, 33 gamma; maize, 100 gamma; oats, 20 gamma; barley, 10 gamma; fresh grass, 50 to 142 gamma; dried grass, 600 gamma; alfalfa meal, 700 gamma; potato, 7.5 to 10 gamma.

It will be noticed that the values given to alfalfa and grass are much higher than those of the cereals. Hunt, Record and Bethke[18] tested alfalfa, clover and timothy hays for vitamin B_2 and found that the content decreased as the plant matured. The value was correlated to the greenness, leafyness and protein content of the plant. Rain, 0.68 in., removed as much as 50 per cent of the vitamin B_2 content. It has been shown recently by Hunt and Bethke[36] that alfalfa exposed to strong sunlight for 48 hours after cutting suffered a loss of about 25 per cent. of its riboflavin content.

The protein supplement forms the principal vehicle for the inclusion of adequate riboflavin in the diet. Milk and yeast are rich sources of the vitamin. The content in milk, although high, will vary according to the diet of the cow.[35] Samples of dried yeast average 2 mg. of riboflavin per 100 grams.

The riboflavin content of meat and meat products is variable. Liver and kidney of pig, cattle, sheep and rabbit contains up to 2 mg. per 100 grams of tissue. The content in muscle is much lower and blood is lacking in the vitamin.

Wilgus *et al.*[17] examined the growth-promoting properties of protein concentrates. They found that in general, growth was due to vitamin B_2 content as well as to the protein. The relative vitamin B_2 content was dried skimmed milk, 19; vacuum dried white fishmeal, 10; meat scrap, 6; soya bean meal, 3; maize gluten, 0; blood, 0.

Christiansen, Deobald, Halpin and Hart,[33] in a study of the effective supplements for soya bean oil meal, noted that the protein of whey was without value as a supplement. It appeared that the value of whole whey was due to its vitamin content. For maximum utilisation of protein in soya bean meal, grain rations, some potent source of flavin and possibly other vitamins must be fed.

Prevention of Curled Toe Paralysis

Riboflavin in its pure form is a water soluble yellowish-green fluorescent pigment. It is unnecessary to use the purified form in the treatment or prevention of the deficiency. Milk and milk products and yeast form the richest and most suitable supplements. Dried skimmed milk at a level of at least 5 per cent. or yeast 3 per cent. should be included in the mash of brooder chickens. If good young grass is not available the addition of 5 per cent. high quality alfalfa meal will supply not only riboflavin but also other necessary vitamins.

Any ration which is composed solely of cereals together with a protein supplement of vegetable origin will contain insufficient riboflavin to promote a maximum growth.

Riboflavin Deficiency in Breeding Hens

Factors influencing the production of eggs of good hatchability are of much importance to the poultry breeder. Many investigations[18, 19, 20] have indicated that a factor or factors other than the fat soluble vitamins are required for hatchability. The factor appeared to be present in the foods now known to be richest in riboflavin, i.e., milk, yeast, liver and fresh green foods. Later work[21, 22] indicated that the required factor was present in the B_2 complex. Bethke *et al.*[23] put forward evidence which indicated that riboflavin was the active factor. Confirmation was made by Lepkovsky *et al.*[24] and by Davis, Norris and Henser.[25, 26]

Insufficient riboflavin in the diet of mature birds does not result in any obvious impairment of health. There is some evidence that egg production, particularly winter production, may be reduced.[24] This would not appear to be marked since Davis *et al.*[25] were unable to note any increase in egg production, fertility, egg size or food consumption when deficient rations were supplemented with riboflavin.

The great importance of riboflavin is its influence upon the hatchability of fertile eggs. Various authors have noted an increase in hatchability of up to 70 per cent. of the total fertile eggs when pure riboflavin was used as a supplement for deficient rations.

Symptoms of Riboflavin Deficiency in Chick Embryos

Little abnormality can be noted in embryos dying before the tenth day of incubation. Embryos dying between the tenth and fourteenth day show markedly retarded growth and are sometimes œdematous.

As incubation proceeds various other defects appear. Lepkovsky[24] has described a degeneration of the embryonic Wolffian bodies, most frequently observed from the 17th to the 21st day. The Wolffian body may be normal in size and filled with a caseous material or swollen to several times its normal

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size and may be œdematous. Scarcity of down and failure of the down feather to rupture its surrounding sheath are not infrequent. The term "clubbed" down is applied to the latter condition. Curling of the toes and embryonic anæmia have been described as symptoms.

Embryonic mortality seems to be highest in the first two weeks of incubation, but as the riboflavin level is increased the mortality shifts towards the later stages of incubation and finally to greatly improved hatchability. There are three peaks of mortality, viz., 1st to 3rd day, 10th to 12th day, and 19th to 21st day.

Many of the chicks which hatch from eggs of hens on a partially deficient ration exhibit symptoms of curled toe paralysis exactly resembling those seen in growing chickens.

Lopkovsky *et al.*[24] noted a lesion of the beak of chicks hatched from the eggs produced by hens on a deficient ration. This occurred at the junction of the horny upper beak and the soft tissue. The lesion appears as a brown necrotic spot. They applied the term "notched" beak and indicated that there was a relationship between the riboflavin content of the hens' diet and the incidence of the occurrence of the lesion.

An examination of the nerves of chick embryos in the eggs of hens on a partially deficient ration showed that myelin degeneration occurred in the sciatic nerve of 60 per cent. of the cases.[27] The incidence of degeneration was less than 10 per cent. when the diet was supplemented with 400 gamma of riboflavin per 100 gms. of food.

Riboflavin Content of the Hen's Egg

It has been shown[28, 29] that vitamin B_2 complex is present both in the egg yolk and the white. This differs from the distribution of vitamin B_1 (thiamin) which is present only in the yolk.

The quantity of riboflavin in the egg depends upon the content in the food.[24, 25, 30] The inclusion of dried skimmed milk, yeast, dried whey, dried liver and certain fish meals in rations low in riboflavin resulted in the production of eggs of increased vitamin content.

The whites of eggs produced by hens on low riboflavin diets are almost colourless; whereas they have a pronounced yellowish-green appearance when the amount of riboflavin is increased.[22, 24] The colour of the white can be taken as a rough index of the riboflavin content of the egg.

Hunt and co-workers[31] have shown that eggs of highest riboflavin content have the highest hatchability, and that chicks from such eggs show the highest livability when fed on a ration deficient in riboflavin. Their results indicate that approximately 220-230 gamma of riboflavin per 100 grams of food are required for production of good hatchable eggs. The riboflavin

requirement for good production appears to be somewhat less than for good hatchability.

Hatchability is influenced in a very short time by changes in the riboflavin content of the ration.[26, 32] During depletion the decline is slight during the third week and greater during the fourth week. The building up of reserves is just as rapid, provided adequate riboflavin is given. Maximum hatchability was obtained during the second week after a deficient ration was supplemented with 900 gamma of riboflavin per hen per week; a longer period was required when the supplement was 600 gamma per hen per week.[26]

Personal Experience

During Spring, 1940, difficulty was experienced in hatching eggs from a flock of in-bred White Leghorn pullets. The breeding pens consisted of six pullets and one male. A grass run was available to each pen. The food used was a commercial mash of unknown composition. A grain mixture of wheat and oats was given daily; oyster shell grit was available *ad lib.*

All the eggs laid were collected and placed in the incubator at weekly intervals.

Fertility was fairly good but the hatchability was consistently bad. The results of three consecutive batches during May were as follows:

Date of hatch	*Fertile eggs*	*Chicks hatched*		*Percentage hatchability*
		Fairly strong	*Weakly*	
15.5.40	127	16	11	21.26
22.5.40	126	9	7	12.70
29.5.40	132	25	9	25.76
Total	385	50	27	20.0

Embryonic mortality during the first two weeks of incubation was 15-20 per cent., but was greater during the third week. Many of the embryos developed fully but were unable to pip the shell or, if they succeeded, were unable to complete the hatching process. The dead embryos were consistently small and were frequently œdematous. Scarcity of down and "clubbed" down were quite common.

The chicks which hatched were small and many did not dry properly. Most were weak on their legs and a number showed the typical stance and gait of curled toe paralysis. "Straddle-legged" birds were not uncommon. About 10 per cent. of the chicks developed slipped tendon whilst still in the incubator. The "notched beak" lesion noted by Lepkovsky was seen quite frequently.

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Observations upon the incidence of this lesion in one hatch (29.5.40) were made:

	Number	*Notched beaks*	*Beaks "clean"*
Strong chicks	25	3	22
Weakly chicks	9	4	5
Shell chipped but hatching not completed	27	7	20
Well-developed embryos dying before chipping shell	55	0	55

It would appear that the notched beak lesion developed at the time of hatching and was probably the result of injury of the soft tissues produced by the chipped rough edge of the shell. The incidence appears to be higher in the weakly chicks which presumably took longer to complete the process of hatching and were thus exposed to the risk of injury by the shell for a longer period.

The symptoms indicated the possibility of riboflavin deficiency in the diet of the hen. Owing to the incidence of slipped tendon it was decided to supplement the ration of half the birds with manganese. Potassium permanganate was added to the drinking water to produce a final dilution of 1/10,000. Riboflavin was not available at the time so dried brewer's yeast was added to the original mash to produce a final content of 4 per cent. (Brewer's yeast contains about 2 mgm. riboflavin per 100 gm.)

Four groups of hens were made up whose food was as follows:

(A) Original food + 4 per cent. brewer's yeast + manganese.
(B) „ „ + 4 per cent. brewer's yeast.
(C) „ „ + manganese.
(D) „ „ No supplement.

The birds were maintained on these foods for ten days before commencing the collection of eggs for incubation. Thereafter the eggs were placed in the incubator at weekly intervals.

The hatchability of the eggs of the groups was as follows:

Group	*Supplement*	*Total fertile eggs*	*Chicks hatched*	*Percentage hatchability*
A	Yeast + manganese ...	173	85	49.5
B	Yeast only	55	36	65.5
C	Manganese only ...	77	15	19.5
D	No supplement ...	141	40	28.4

The addition of yeast resulted in considerable improvement of hatchability and in the quality of the chicks hatched. This experiment does not afford proof that the riboflavin factor in the yeast was responsible for improved hatchability, but in the light of previous work it is indicative.

The hatchability, although increased, did not reach the level expected in good strains. The cause is not clear. It may be the result of close inbreeding of the strain over a number of years or because an insufficient period was allowed for the building up of reserves of the factor in the body and of passing it on to the egg before the experiment was commenced.

The addition of manganese did not result in any improvement. In view of the small numbers of eggs no significance can be ascribed to the fall in hatchability following the addition of manganese, i.e., in groups A and C.

Prevention of Riboflavin Deficiency in Breeding Birds

If soya bean meal, earth nut meal, cotton seed meal are used as protein supplements in the mash of breeding birds they should be combined with some product rich in riboflavin; of these, the best are dried milk or yeast. Meat and fish meals vary in their riboflavin content and cannot be relied upon to supply an adequate amount. If good young growing grass or clover is available, deficiency is unlikely to be severe. If, however, the range is poor an addition of 5 per cent. alfalfa meal to the mash should be made.

Summary

Deficiency of riboflavin in the food of growing chickens results in poor growth and a characteristic form of leg weakness known as curled toe paralysis.

Inadequate riboflavin in the diet of breeding hens results in loss of hatchability. This condition can be prevented by including some product rich in riboflavin.

The best sources of riboflavin are milk and milk products, yeast, liver meal and fresh and dried green foods.

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Clinical Articles

GRASS DISEASE IN A COAL MINE

By A. A. FORSYTH, M.R.C.V.S., D.V.S.M.

EARLY in June, 1927, a mysterious disease appeared among ponies below ground in a local colliery. A pony was reported to be off his feed and had passed no dung for twelve hours. He showed no acute abdominal pain, but was sweating in patches, temperature normal, and the pulse was hard and wiry. He had great difficulty in swallowing. The buccal and rectal membranes were sticky to the touch and the large bowel was full of hard fæces. He had the appearance of a horse extremely ill; his anxious look and tucked-up abdomen will not be easily forgotten. He was treated with aloes and enemata and he died within twenty-four hours of his return to the stable from work. A second and a third pony showed similar symptoms and died in about the same time.

Post-mortem showed nothing abnormal anywhere except in the large intestine, which in all cases was full of hard, dry fæces which separated from the bowel wall only with difficulty.

It was not until the third pony had died that I had any idea of what the condition might be, and by this time two more ponies were ill in another pit belonging to the same firm. The head horsekeeper, a very intelligent man, noticed that the first symptom was sweating under the tail, even though the

horse was eating. This was taken as a guide to all horsekeepers, who examined every horse in the stable at least twice during his shift and noticed how much dung was passed. Drivers were warned to return any horse to the stable at half time which had not passed dung while at work.

Every horse which had not dunged during a period of four hours was dosed with a pint of liquid paraffin and brought to the surface at the end of the shift. In all, fourteen horses were brought to the surface. Six were false alarms and eight were affected. Seven died and one recovered. With the exception of the one which recovered, the longest that any pony lived after the first symptoms was four days.

The pony which recovered was " Cobbler," a dark bay five-year-old Welsh pony gelding, about 12 hands. He was returned to the stable by his driver after half a shift's work because he was " tired to death " and had not passed dung. He was sweating under the tail. He was given a pint of liquid paraffin and brought to the surface at the end of the shift. He was given more paraffin and enemata of glycerin and water. Linseed tea sweetened with treacle was the only thing he would swallow voluntarily and this was given *ad lib.* during the whole course of the illness. By this time my stock of liquid paraffin was exhausted, as was that of the local chemists'. The colliery engineer provided a very efficient substitute in the form of " dynamo oil," a pure hydrocarbon of slightly higher specific gravity than B.P. paraffin liq., straw-coloured, almost tasteless, and with no free carbon.

This pony had over five gallons of dynamo oil before there was any motion of his bowels, which did not move for a week, although there was a leakage of paraffin from the anus on the fourth day. After a week the patchy sweating gradually subsided and in a fortnight he was able to eat solids and swallow normally. He was kept in a loose-box and exercised and returned to work in the pit after an absence of eight weeks. He is now eighteen years old, in good condition and working regularly. Great care is still taken with his diet, an extra allowance of bran in summer and roots in winter is sent down for him every day. Should it be missed for one day constipation immediately follows, and a bottle of liquid paraffin is kept specially for him. Since his return to the pit he has not missed a single day's work for any other cause than constipation, which is not accompanied by abdominal pain but when it occurs it is very persistent.

The only common factor between the two pits affected was food, and after the death of the third pony no more foodstuffs in the corn stores were used. Hay from the colliery farm was chaffed there and mixed with home-grown oats and locally milled bran. The corn stores were closed and thoroughly " spring cleaned," disinfected and whitewashed and the machinery overhauled. The corn sacks were taken to the boilers and sterilised by superheated steam each time they came from the pit before being refilled. In the pits the corn boxes used in the workings were brought to the surface with any corn that

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could be gathered from the ground and burned. The mangers in the stables were scrubbed with a hot 5 per cent. solution of washing soda, and the standings whitewashed.

Of the foodstuffs used at the beginning of the trouble, the oats and beans were grown locally and the bran came from a nearby mill. The hay came from a dealer in the South of England who had sent a canal barge of hay in exchange for a cargo of coal. After long and wide inquiries I found that this hay was part of a consignment which had been returned to the dealer by a large London stud after their horses had shown symptoms similar to those seen here.

When the corn stores were cleaned out the whole of the foodstuffs were fed to cattle on the colliery farm and although a careful watch was kept over them no symptoms of any stomach or bowel derangement were ever noted.

A CASE OF TUBERCULAR MENINGITIS IN A STIRK

By PHYLLIS STUBBS, M.R.C.V.S.,
and
D. O. SUTTON, B.V.Sc., M.R.C.V.S.
Preston.

THE subject was a red and white cross-bred Shorthorn stirk, aged about ten months. The owner rang up on Monday, November 4, to say that this animal had been discovered lying stretched out in the loose-box. On the Sunday evening, when fetching up his young stock, this stirk had been found away from the others feeding from the bottom of a wide, damp ditch. While she was being driven home, the owner noticed that she had difficulty in walking, appearing stiff in the limbs and reeling badly. She was given immediately about half a pound of magnesium sulphate.

When the writers saw her the following morning the stirk was lying on her right side, with the limbs rigidly extended, back hollowed, and neck bent backwards: it was impossible to flex the neck by force. Periodically the limbs were flexed, but extended again immediately. The temperature was 102.5 deg. F., respiration was laboured and rapid: there was no tympany. Any pressure applied over the spine produced strychnine-like convulsions.

In view of the symptoms and history, poisoning was suspected. The ditch in which the animal had been grazing was examined, and water dropwort (*Œanthe crocata*) was found growing there in abundance. In his "Veterinary Toxicology" Lander describes similar nervous symptoms to those noted above occurring in poisoning by the roots of this plant.

A tentative diagnosis of poisoning by dropwort was given, and prognosis was very unfavourable. The stirk was drenched with linseed oil and turpentine, and instructions left that she should be turned at intervals.

The condition remained unchanged throughout the first and second days. On the third day it was found that she was able to rise with assistance from her left side, but she was unable to move at all if on her right side. Milk was taken readily and also a little calf cake, although she had to be supported while feeding. By the fifth day she had made further improvement, and was able to get up without assistance from her left side, but was still helpless when on her right side.

The stirk remained in this same condition until six days later when she was unable to rise at all, or even to support herself when lifted. The condition became worse, the convulsions appearing again, and twelve days after the illness had started she was destroyed.

A post-mortem examination revealed miliary tuberculosis of the lungs, and tuberculous lesions in the following sites: bronchial and mediastinal glands, mesenteric glands, liver and peritoneum. The submaxillary and retropharyngeal glands were also affected, and the brain and upper cervical portion of the spinal cord were examined. Both the brain and spinal cord had been damaged during removal, but no macroscopic changes were noted. Smears were made from the cerebral and spinal meninges, and also from the brain substance from a section made at the level of the cerebellum. In all these smears acid-fast organisms similar to tubercle bacilli were found.

This was the only stirk on the farm which had not been bred by the owner. Throughout the case she showed no clinical symptoms of tuberculosis, being in good bodily condition, and only on one occasion was a slight cough noted.

PERSISTENT THYMUS IN MILKING COWS

By A. A. FORSYTH, M.R.C.V.S., D.V.S.M.

After attending post-mortem examinations for over twenty years I have only seen three cases of persistent thymus in adult cattle.

I can find no mention of it in the literature at my immediate disposal. Had I realised at the time that the condition was so rare, great care would have been taken to preserve the glands for further investigation.

Case 1.—A red Shorthorn cow had calved her third calf about four and a half months and had been served again about a month. She was giving between four and five gallons of milk per day. On the morning of her death she gave her usual amount of milk and ate all her food. She was found dead in the stall on the return of the attendants from breakfast. The neck chain was released without difficulty and there were no signs of any struggle.

The blood was examined for anthrax, which proved negative, and the carcase was removed to a knackery.

Careful examination of abdominal and thoracic contents revealed neither abnormality nor macroscopic lesions of disease. In the lower third of the neck ventral to the trachea was a thymus gland similar in appearance and consistence to a parotid. It was about eight inches long, four and a half inches broad, and about half an inch thick. The thorax was searched carefully for any remains of a thoracic portion but none was found. Higher in the throat was some slight bruising, as if the neck chain had been too tight; the hyoid bone was not broken nor were any of the tracheal rings. As the lungs showed none of the signs of suffocation, this seemed too trivial to be the cause of death of a healthy animal.

Case 2.—A pedigree Jersey cow, newly purchased, had arrived on the farm a week before death. She had calved her second calf which was a week old, and appeared very strong, and the calf stood the journey from Hertfordshire by road better than her mother, which suffered from indigestion for two days after arrival, but recovered. I saw her casually while attending another case on the day before she died, when she looked worth the high price paid for her.

On the morning of death she milked as usual, ate her food and was apparently normal at 9 a.m. At 10 a.m. I found her lying on her brisket with a hind leg on either side of the abdomen, the head was erect and the ears dropped back; she remained in this position after death. Temperature was normal, pulse and respiration almost imperceptible. I heard her heart slow and stop through a stethoscope. No treatment was attempted as she was obviously dying.

Post-mortem.—The peritoneal, pleural and pericardial sacs were filled to capacity with a lymph-like fluid and although twenty-four hours had elapsed between death and post-mortem examination there was no sign of clotting or fibrinous deposit. A direct smear stained with methylene blue showed a few leucocytes but no bacteria. The abdominal contents were carefully examined for any inflammatory condition, but none was discovered in either alimentary or urogenital system. In the thorax both lungs were collapsed but were readily inflated; no inflammatory condition was discovered. On the floor of the chest anterior to the heart, reaching to the entrance, was a thymus gland about one and a quarter inches in thickness, almost square in shape, with sides of about six inches. The neck was examined for a cervical portion but none could be found.

Case 3.—Of this case I was unable to trace any history. I found her being dressed in a knackery which I had visited to see the carcase of another animal. She was a red Shorthorn cow about four years old, in milk, with a fœtus of about four months in utero, one ear was missing, so that it can be presumed that she had died suddenly.

There was a remarkable similarity between the post-mortem findings in this carcase and in that of Case 2. When the abdomen was opened a large quantity of clear straw-coloured fluid escaped and here again there were no clots of fibrin; no inflammatory changes were found in the alimentary or urogenital systems and there was no excess of fluid in the uterus. The pericardial and pleural sacs were filled with similar fluid, both lungs were collapsed and were easily inflated with a tyre pump. There appeared to be no inflammatory changes. On the floor of the chest was a thymus gland somewhat triangular in shape with its base posteriorly about nine inches long and five inches wide at its base, tapering to two inches at its apex. The lobulation was larger than that of a normal calf's thymus. There was no cervical portion.

All the animals were in good bodily condition, none showed lesions of tuberculosis or fluke. There may be some analogy between Case 1 and the so-called status thymus lymphaticus seen in the human subject. Case 2 was the only one I saw alive and then only for a few minutes. Death was no doubt due to pressure on the heart and lungs of an extremely sudden effusion from the serous membranes, the cause of which I do not know. The similarity of the post-mortem findings in Cases 2 and 3 are worthy of note. With the present lack of knowledge of thymic function, I would hesitate to give an opinion as to whether the presence of a thymus contributed in any degree to the death of any one of these cows.

FROM A PRACTITIONER'S NOTEBOOK

By C. S. HASELDEN, M.R.C.V.S.

Northwood, Stoke Mandeville, Bucks.

Subject.—Flock of 200 in-lamb ewes.

History.—Professional advice regarding this flock was sought on December 17, 1939, as some 30 ewes had aborted. The breeding of this flock is unique, and their value considerable. The ewes were aborting at the rate of two a day. A provisional diagnosis of Salmonella abortus ovis infection was made, prognosis being grave. This organism was isolated later from two aborted fœtuses by Dr. Hare.

Treatment.—An autogenous vaccine was prepared and was administered to the entire flock in two doses of 5 and 10 c.c. per ewe, at a week's interval. Up to the third week following the first dose of vaccine a total of 68 ewes had aborted. Thereafter no further abortion occurred among the remaining 132 ewes, which produced and reared 208 lambs.

All the ewe lambs kept for breeding purposes were inoculated in a similar manner at the beginning of last September. It is interesting to note they are an extremely promising and well-developed crop, and that the ewes which

actually aborted last year have this year carried their lambs to the full time, some 50 lambs having been born by January 8, 1941.

Subject.—Pedigree Shorthorn cow.

History.—On May 27, 1940, an urgent call was received. It referred to a cow which had been taken suddenly very ill.

Diagnosis.—On arrival at the farm it was found that the cow had a temperature of 107 deg. Respirations were rapid, shallow and entirely abdominal. The animal refused all food. Auscultation of the chest revealed the complete absence of air sounds, and a diagnosis of acute congestion of the lungs was made.

It is perhaps worth noting that a differential diagnosis between acute congestion of the lungs and traumatic pericarditis had to be made. Traumatic pericarditis was ruled out as there was no previous history of tympanitis of the rumen, absence of a localised area of pain on percussion of the pericardial area and œdema of the dewlap, and the fact that the heart beats were distinct and unmuffled.

Treatment.—A plaster of kaolin poultice was applied to the chest wall and covered with brown paper; the animal was rugged up and placed in a warm but airy box. An intramuscular injection of 30 c.c. of a 20 per cent. solution of sulphonamide (May & Baker's Soluseptacine) was given. A similar daily injection of sulphonamide was given for the next three days. The temperature fell by a degree each day. The kaolin poultice was renewed every 24 hours. During this time the animal ate practically nothing and the secretion of milk ceased. By June 3 the temperature had dropped to 102 deg., air sounds in the lungs were gradually returning, and the animal appeared much brighter, but still refused practically all food. A further injection of sulphonamide was given. By June 6 the temperature was normal, air sounds had returned fully to the lungs, and the secretion of milk had commenced again, but inappetance persisted. A course of simple tonic powders containing nux vomica and sodii bicarb. was given. It was decided to dry the cow off.

The animal soon regained the weight she had lost and is now perfectly normal.

Subject.—A herd of cross-bred Friesian-Shorthorn cows.

History.—The first visit to this herd was made on February 26, 1940, to attend a cow suffering from acute mastitis.

Diagnosis.—The animal was extremely ill; inappetance, temperature 106 deg., the entire udder was much enlarged, hard and inflamed. Only a small quantity of milk could be obtained from the near fore quarter. A sample of mammary exudate was sent to Dr. Hare for bacteriological examination.

Treatment.—An intramuscular injection of 30 c.c. of 20 per cent. sulphonamide (May & Baker's Soluseptacine) was given immediately, the udder

fomented and stripped three or four times a day, and afterwards greased. The next day another injection of sulphonamide was given. On February 28 5 c.c. of a stock mastitis vaccine, prepared by Dr. Hare, together with 30 c.c. of sulphonamide, was given, it being thought that the one would have a synergic action on the other.

Bacteriological examination of the mammary exudate revealed a pure culture of *Straphylococcus aureus,* and an autogenous vaccine was prepared and was injected in doses of 5 c.c. at 48-hour intervals until a total of 30 c.c. had been given. The injections of sulphonamide were continued until March 4, by which time the temperature had returned to normal, and the condition of the udder greatly improved. The only quarter which had not returned to normal was the off fore. It is interesting to note how rapidly an attack of acute mastitis will cause loss in bodily condition. This cow was perhaps on the fat side when taken ill, but within a week she was literally "skin and bones."

Two days after the onset of mastitis in this beast another cow developed a sub-acute attack. Bacteriological examination of the milk revealed a pure culture of the *Alpha Hæmolytic Streptococcus.* Two more cows became similarly affected soon afterwards and it was decided to inject into the 21 animals in the herd an autogenous vaccine prepared from the staphylococci and streptococci already isolated.

The vaccine was administered in doses of 5, 10 and 15 c.c. at a week's interval and a further three doses were administered three months later, since which time there has been no further outbreak.

Of the four cows which showed acute symptoms of mastitis three have lost partially the use of one quarter.

The author has not been impressed by the results following irrigation of the udder and no longer practises this technique.

A CASE OF ŒSOPHAGEAL OBSTRUCTION ON TWO OCCASIONS IN SAME ABERDEEN TERRIER

By G. DUNLOP MARTIN, M.R.C.V.S.
Shenfield.

First occasion, seven years ago.

A three-year-old male Aberdeen terrier had been seen by another veterinary surgeon 14 days previously. X-ray pictures had been taken showing a large foreign body in the thoracic part of the œsophagus adjacent to the curve of the aorta.

An anæsthetic was given and a probang passed which located an obstruction where shown in X-ray. Another X-ray was taken and confirmed previous one. An operation being advised, the owner said: "The dog would be sure to die." My answer was: "He will die if operation is not attempted. Give him his chance."

Operation (16 days later).—The œsophagus was exposed and opened at the base of neck. The foreign body was grasped with long forceps and disengaged with difficulty. On bringing it into wound it had to be divided by bone forceps into five pieces to facilitate removal. The pieces were very foul-smelling. Fitted together they formed the upper epiphysis of a sheep's tibia which must have passed down the œsophagus on edge in order to go between two first ribs. The dog made most happy and uninterrupted recovery.

In May, 1940, I saw the same dog again with symptoms suggesting a foreign body. X-ray showed a foreign body in practically the same position as that previously described.

At operation it was impossible to dislodge the body through the operation incision in the base of neck, so laparotomy and gastrotomy were performed. The body was grasped but could not be pulled down into stomach and so the dog was destroyed. The foreign body proved to be portion of occipital bone of a sheep.

Another case of foreign body in stomach of seven-year-old Aberdeen terrier male, belonging to same owner.

When consulted re this dog in August last the owner said he had been losing condition very gradually for some months, though eating normally. It had been noticed he had been sick first thing in the morning, perhaps once in four or five days, *never* bringing up food but only a very little yellowish frothy liquid. On the idea of a foreign body being suggested to the owner this was scouted, as they were so very careful owing to what had happened in the case previously described. However, an X-ray was taken and showed *two* foreign bodies in the stomach. By this time the dog was *thin*—definitely. Gastrotomy disclosed the stomach to be considerably dilated and the musculature wasted; in fact, the stomach wall generally was very thin.

On opening the stomach the two foreign bodies proved to be the halves of a *rubber Mickey Mouse.*

The stomach walls proved unequal to holding sutures so the dog was destroyed.

The extraordinary, in fact *astounding,* thing about the case is that the Mickey Mouse had disappeared from its usual place (the dining-room mantelpiece in *August,* 1939, and was presumed to have fallen into the fire or been thrown out unnoticed in the sweepings from the room. The dog must have swallowed it a year previous to my seeing him, which one would be inclined to think incredible!

Abstracts

THE CONTROL OF BANG'S DISEASE BY SLAUGHTER

By W L. WILLIAMS

Veterinary Medicine, XXXV, 8, August, 1940.

THIS article falls under three heads: (i) a brief review of the various bacteriological methods which have been advocated to control the disease; (ii) a plea for the fuller recognition and adoption of rational methods of animal husbandry; and (iii) a discussion of the United States plan for the control of abortion by the slaughter of reactors to the agglutination test.

The author refers to the use of vaccines, both dead and living, in pregnant and non-pregnant animals, and to calfhood vaccination with feebly virulent material. He points out that all these measures have met with early success, but that further experience has not supported the claims made for them. Williams contends "the experimenters fail to record essential data and lead the reader to erroneous conclusions." He makes the further comment ". . . no chronic disease has yet been controlled by quarantine. . . . Neither has any chronic infectious disease been controlled by artificial immunisation in such pronounced manner that this method has been generally accepted." Vitamins and endocrine preparations have been advocated by some writers. The author finds it difficult to understand why a healthy cow should be unable to secure adequate vitamins from good food by the ancient process of digestion, nor why "a healthy cow with a normal complement of ductless glands must have endocrines extracted from the ductless glands of a mule in order to produce a healthy calf."

The theme of the author's advice is that abortion should be controlled "by the natural process of raising the resistance of the animals through conservative breeding, feeding, and handling." He maintains it is well known that in virtually all contagious diseases some individuals fail to become diseased. In one herd 1 per cent. to 5 per cent. of the cows may react to the agglutination test, in another 80 per cent. to 100 per cent. react. In one herd the power of resistance is high, in the other it is low. Or if one prefers it, the virulence of the organism is low in the former instance and high in the latter. Moreover, the highest incidence of genital pathology, including abortion, occurs in large dairy herds, maintained under high pressure of breeding, feeding, and handling for the production of the maximum quantity of milk.

The writer is firmly of the opinion that so long as these unnatural conditions obtain, so long also will abortion continue to flourish.

Commenting on the official policy of the Bureau of Animal Industry—the slaughter of all animals positive to the agglutination test—Williams contends that the results are far less satisfactory than appears to be the case from a superficial examination of the data. He discusses the position in the different States, giving reasons why some States have a much higher percentage of their cattle under official control. (The plan is a voluntary one, apparently without compensation for slaughtered animals.) A comparison between the States of New York and Wisconsin will serve to illustrate his argument. New York is pre-eminently an industrial State consuming large quantities of fluid milk. Because of vast areas of unfertile soil, hills and mountains, it cannot grow sufficient grain and hay to feed its cattle, but must purchase most of its grain and protein food, and much of its hay from the Mississippi Valley. For economic reasons the cows are largely bred to calve during the last quarter of the year. The vigour of the cows is much depressed. The fate of the calves is far worse. Deprived of sunlight and fresh air and badly fed, there is great mortality from calf scour and pneumonia. The survivors are largely weaklings devoid of vigour until at least six months old, when ovulation and spermatogenesis become established, and the quality of sexual development has become fixed for life. A brief nine months elapses and the half-grown heifer is bred, with great probability that she will abort, suffer from dystokia, retained afterbirth, metritis or other pathological phenomena. Under these conditions the New York herd cannot maintain itself. It must purchase cows to replace those discarded for genital disease. Many of these cows come from Wisconsin. Williams observes that in New York less than 5 per cent. of the cattle are under official control. The slaughter of reactors to the agglutination test often means bankruptcy to the dairyman, hence where the need for control is greatest it cannot be undertaken for financial reasons. Thus far neither slaughter nor any other plan has yielded acceptable results.

In Winconsin conditions are very different. The State produces mainly butter and cheese. Cows may, therefore, be bred when in maximum vigour, while at pasture in the early summer, and the calves may be born at the natural season in the spring. They in their turn have the advantage of summer and succulent grass during the first months of life. Further, Wisconsin is a highly fertile agricultural State. Accordingly there is produced a surplus of highly efficient dairy cows. New York State requires that cattle entering from other States shall be negative to the agglutination test. Hence the enhanced value of the cows sold more than compensates the Wisconsin farmer for the loss of reactors slaughtered under the control plan. So Wisconsin has 35.6 per cent. of its cattle under Federal State control. The need for control is, however, far less than in the case of New York. Williams submits that the position is just the reverse of the relative public health and economic needs

for the control of genital disease in cattle. The concluding sentence of the article is worthy of reproduction in full. "But since other methods have failed may it not occur soon that veterinarians and animal husbandmen will pause and give thoughtful study to the possible value of the conservative and rational breeding, feeding and handling of domestic animals as the foundation for physiological reproduction?" Such strongly expressed opinions may not meet with universal acceptance, even in the author's own country. They do, however, command respect and unbiased consideration, for, to quote *Veterinary Medicine*, "Whether or not one agrees with Prof. Williams one must agree that he is No. 1 man in this country in knowledge of and experience with breeding diseases of animals."

Reviews

Veterinary Hygiene. By R. F. LINTON, Ph.D., M.R.C.V.S., Royal (Dick) Veterinary College, Edinburgh (1940). Pp. 487. 127 Figs. Numerous Refs. Edinburgh: W. Green & Son, Ltd. 3rd Edit. Price 25s.

THIS is a revised edition of the well-known book of the Edinburgh Veterinary Series. The general lay-out of the book is similar to the previous editions. The number of sections has been reduced from six to five by the omission of the section of Metereological Phenomena and Instruments, but the essentials have been incorporated in the section dealing with Air and Ventilation.

In the section dealing with the control of disease, the chapters concerning "Notifiable Diseases" have been largely re-written and brought up to date consequent upon the changes created by the formation of the State Veterinary Service under the Agriculture Act of 1937. A clear and concise account of the present machinery is given. In the same section there are additional chapters on the Tuberculosis (Attested Herds) Scheme, Johne's disease and on certain diseases of poultry. The recent Orders concerning poultry are also discussed.

Elsewhere the book remains substantially the same as in the previous edition, but there are small useful additions concerning such subjects as the disposal of farmyard manure, the "jetting" of sheep for blow-flies and the outdoor penning of sows and young pigs.

The book, whilst containing little new knowledge, will still be acknowledged as the standard English textbook on veterinary hygiene.

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Diseases of Poultry: Their Ætiology, Diagnosis, Prevention and Control, with a Section on the Normal Anatomy and Physiology of the Fowl. By ERNEST GRAY, M.R.C.V.S., F.R.S.M. Pp. 198. 39 Illustrations. London: Crosby Lockwood & Son, Ltd. Price 9s. 6d.

WE read this book with much interest for it is well known that the author has devoted a considerable amount of time in recent years to the study of poultry diseases. The theme of the book can be briefly stated by quoting from the preface: "Only hygiene and sanitation based upon common sense will preserve the poultry flocks of the future; and it is the duty of every poultry keeper to educate himself to this end." The book was written presumably to assist the poultry farmer "to educate himself to this end."

There is a preface 16 chapters and an index and the sequence is anatomy and physiology, hygiene and sanitation, health and disease, general pathology, special pathology, specific diseases, parasitic diseases and a chapter on the practical application of the work.

While on the whole the author may be said to have achieved his object in making the poultry keeper "disease-minded" and in imparting to him his (the author's) opinions and beliefs concerning the prevention and control of poultry diseases, it is to be regretted that errors have appeared and, in some instances, the statements may not be generally accepted. To do justice to such an extensive subject the author would require to expand the work considerably. The sections dealing with disease are useful and, in most, there is to be found information of value to those interested in diseases of poultry. The illustrations are good and the work is well produced.

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